

WHAT IS CLAIMED IS:

1. A method for identifying markers for a human disease state, comprising the following steps:

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- a) providing human peripheral blood mRNAs;
- b) amplifying said mRNAs to provide nucleic acid amplification products;
- 10 c) separating said nucleic acid amplification products; and
- d) identifying those mRNAs that are differentially expressed between normal individuals and individuals exhibiting said disease state.

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2. The method defined in claim 1, further described as comprising the step of converting said RNAs into cDNAs using reverse transcriptase.

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3. The method defined in claim 2, further comprising using random hexamers, arbitrarily chosen oligonucleotides, promiscuous oligonucleotide primers or anchoring primers for the reverse transcription step.

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4. The method defined in claim 3, further comprising using said arbitrarily chosen oligonucleotides, promiscuous oligonucleotide primers and/or anchoring primers for the amplification step.

5. The method defined in claim 1, wherein the disease state is metastatic or organ confined cancer, asthma, lupus erythromatosis, rheumatoid arthritis, multiple sclerosis, myasthenia gravis, autoimmune thyroiditis, ALS (Lou Gehrig's disease), interstitial cystitis or prostatitis.

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6. The method defined in claim 5, wherein the disease state is metastatic prostate cancer.

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7. The method defined in claim 5, wherein the disease state is metastatic breast cancer.

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8. A method of detecting a human disease state, comprising the steps of:

a) detecting the quantity of a disease marker expressed in human peripheral blood; and

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b) comparing the quantity of said marker to the quantity expressed in peripheral blood of a normal individual;

wherein a difference in quantity of expression is indicative of a disease state.

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9. The method of claim 8, wherein said disease marker is an mRNA.

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10. The method of claim 9, wherein said mRNA is amplified by an RNA polymerase reaction.

11. The method of claim 9, wherein said mRNA is amplified by reverse transcriptase polymerase chain reaction or the ligase chain reaction.

5 12. The method of claim 8, wherein said detecting is by RNA fingerprinting, branched DNA or a nuclease protection assay.

10 13. The method of claim 8, wherein the disease state is metastatic cancer, asthma, lupus erythromatosis, rheumatoid arthritis, multiple sclerosis, myasthenia gravis, autoimmune thyroiditis, ALS (Lou Gehrig's disease), interstitial cystitis or prostatitis.

15 14. The method of claim 8 wherein the disease state is metastatic cancer.

15 15. The method of claim 14 wherein the metastatic cancer is metastatic prostate cancer.

20 16. The method of claim 14 wherein the metastatic cancer is metastatic breast cancer.

25 17. The method of claim 9 in which said mRNA comprises one or more of the sequences or the complements of the sequences disclosed herein as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:29, SEQ ID NO:34, SEQ ID NO:48 or SEQ ID NO:49.

18. The method of claim 8 in which said marker is a product of an interleukin 8 (IL-8) or interleukin 10 (IL-10) gene.

5 19. The method of claim 9, further comprising the steps of

a) providing primers that selectively amplify said disease state marker;

b) amplifying said nucleic acid with said primers to form nucleic acid
10 amplification products;

c) detecting said nucleic acid amplification products; and

d) measuring the amount of said nucleic acid amplification products formed.
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20. The method of claim 19 in which said primers are selected to specifically amplify a nucleic acid having a sequence comprising SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:29, SEQ ID NO:34, SEQ ID NO:48 or SEQ ID NO:49.
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21. The method of claim 8, wherein said marker is a polypeptide.

25 22. The method of claim 21, wherein said polypeptide is encoded by a nucleic acid sequence comprising the sequence disclosed herein SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:29, SEQ ID NO:34, SEQ ID NO:48 or SEQ ID NO:49.

23. The method of claim 21, wherein said detection is by an antibody immunoreactive with said marker.

5 24. The method of claim 21, wherein said polypeptide is encoded by an IL-8 or IL-10 gene.

10 25. The method of claim 8, wherein said marker is a product of the IL-8 gene and wherein said comparison is between two alternatively spliced forms of an IL-8 gene product.

15 26. The method of claim 24, wherein the quantity of IL-8 polypeptide in peripheral blood is measured using an *in vitro* bioassay that detects an IL-8 mediated biological process.

20 ~~27. A method for treating a subject with cancer comprising the steps of:~~
~~(i) providing an antisense expression construct comprising a nucleic acid, wherein the RNA expressed from said nucleic acid binds under high stringency conditions to a nucleic acid comprising SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:29 or SEQ ID NO:34, and a promoter functional in eukaryotic cells wherein said nucleic acid is under transcriptional control of said promoter; and~~
~~25 (ii) contacting said expression construct with peripheral leukocyte cells of said subject in a manner that allows the uptake of said expression construct by said cells.~~

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28. The method of claim 27, wherein expression of said nucleic acid results in the modification of the immune response of said peripheral leukocyte cells to said cancer.

29. The method of claim 25 wherein said markers comprise SEQ ID NO:2 and SEQ ID NO:3.

~~30.~~ A kit for use in detecting a human disease comprising:

(a) a pair of primers for amplifying a disease state marker consisting of a nucleic acid; and

(b) containers for each of said primers.

31. A kit according to claim 30 in which the pair of primers is selected to amplify a nucleic acid marker for metastatic human cancer.

32. A kit according to claim 31 in which the pair of primers is selected to amplify a nucleic acid having a sequence comprising SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:29, SEQ ID NO:34, SEQ ID NO:48 or SEQ ID NO:49.

33. A kit according to claim 31, comprising:

(a) a pair of primers selected to amplify a nucleic acid sequence comprising SEQ ID NO:2; and

(b) a pair of primers selected to amplify a nucleic acid sequence comprising SEQ ID NO:3.

5 34. A kit for use in diagnosing metastatic cancer in a biological sample, comprising:

(a) an antibody which binds with high specificity to a polypeptide having an amino acid sequence encoded by a nucleic acid sequence comprising SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:29, SEQ ID NO:34, SEQ ID NO:48 or SEQ ID NO:49; and

(b) a container for said antibody.

15 35. A kit according to claim 32, further defined as comprising:

(a) an antibody that binds with high specificity to a soluble IL-8 gene product;

(b) an antibody that binds with high specificity to a membrane bound IL-8 gene product; and

(c) a container for each antibody.

25 36. A kit according to claim 34, wherein said metastatic cancer is metastatic prostate cancer.

37. A method of detecting prostate cancer in a biological sample, comprising:

(a) measuring the levels of IL-8 or IL-10 in combination with at least one prostate disease marker in said sample; and

(b) comparing said levels with corresponding levels obtained from reference populations of normal individuals, individuals with BPH and individuals with prostate cancer.

38. The method of claim 37 in which said prostate disease marker is selected from a group consisting of total prostate specific antigen (PSA); prostate specific membrane antigen (PSMA=Folic Acid Hydrolase); prostate acid phosphatase (PAP); prostatic secretory proteins (PSP₉₄); human kallekrein 2 (HK2); and the ratio of the concentrations of free and bound forms of PSA (f/t PSA).

39. The method of claim 38, in which the biological sample comprises peripheral human blood.

40. The method of claim 39, wherein the level of IL-8 in a biological sample is measured using at least one antibody that binds to an IL-8 gene product.

41. The method of claim 40, wherein the level of IL-8 gene product bound to antibody is measured by ELISA.

42. The method of claim 39, wherein the level of IL-8 in a biological sample is measured using at least one oligonucleotide probe that binds to an IL-8 messenger RNA (mRNA).

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43. The method of claim 42, wherein an IL-8 mRNA is alternatively spliced to include intron 3.

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44. The method of claim 42, wherein the level of oligonucleotide probe bound to IL-8 mRNA is measured by nuclease protection assay.

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45. The method of claim 42, wherein the level of oligonucleotide probe bound to IL-8 mRNA is measured by RT-PCR™.

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46. The method of claim 42, wherein the level of oligonucleotide probe bound to IL-8 mRNA is measured by ligase chain reaction.

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47. The method of claim 42, wherein the level of oligonucleotide probe bound to IL-8 mRNA is measured by PCR™.

48. The method of claim 39, wherein the level of IL-8 in a biological sample is measured using an *in vitro* bioassay that detects an IL-8 mediated biological process.

49. The method of claim 39, wherein the level of IL-8 in a biological sample is measured using at least one molecule that binds to an IL-8 gene product, wherein said molecule is selected from a group consisting of: an IL-8 binding protein; and an IL-8 receptor protein.

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50. The method of claim 38, wherein the level of prostate disease marker in a biological sample is measured using at least one antibody that binds to at least one prostate disease marker protein.

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51. The method of claim 50, wherein the level of prostate disease marker protein bound to antibody is measured by ELISA.

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52. The method of claim 38, wherein the level of prostate disease marker in a biological sample is measured using at least one oligonucleotide probe that binds to at least one prostate disease marker messenger RNA (mRNA).

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53. The method of claim 42, wherein the level of oligonucleotide probe bound to prostate disease marker mRNA is measured by nuclease protection assay.

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54. The method of claim 42, wherein the level of oligonucleotide probe bound to prostate disease marker mRNA is measured by RT-PCR™.

55. The method of claim 42, wherein the level of oligonucleotide probe bound to prostate disease marker mRNA is measured by ligase chain reaction.

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57. The method of claim 42, wherein the level of oligonucleotide probe bound to prostate disease marker mRNA is measured by PCR™.

5 58. A method of differentially diagnosing prostate cancer and benign prostatic hyperplasia comprising the step of measuring the levels of IL-8 or IL-10 in combination with at least one prostate disease marker in a biological sample.

10 59. The method of claim 58 in which said prostate disease marker is selected from a group consisting of: total prostate specific antigen (PSA), prostate specific membrane antigen (PSMA=Folic Acid Hydrolase), prostate acid phosphatase (PAP), prostatic secretory proteins (PSP₉₄), human kallekrein 2 (HK2), and the ratio of the concentrations of free and bound forms of PSA (f/t PSA).

15 60. The method of claim 58, in which said biological sample consists of peripheral human blood.

20 61. A kit for detecting or differentially diagnosing human prostate cancer comprising:

(a) at least one detection agent for measuring the levels of IL-8 or IL-10 in a biological sample;

25 (b) at least one detection agent for measuring the levels of at least one prostate disease marker in said biological sample; and

(c) containers for each of said detection agents.

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62. The kit of claim 61 in which said prostate disease marker is selected from a group consisting of total prostate specific antigen (PSA), prostate specific membrane antigen (PSMA=Folic Acid Hydrolase), prostate acid phosphatase (PAP), prostatic secretory proteins (PSP₉₄), human kallekrein 2 (HK2), and the ratio of the concentrations of free and bound forms of PSA (f/t PSA).

63. The kit of claim 62 in which said detection agents are selected from a group consisting of polyclonal antibodies; monoclonal antibodies; oligonucleotides; paired oligonucleotides designed to bind to opposite strands of a double-stranded DNA molecule; and at least one molecule that binds to an IL-8 gene product.

64. The method of claim 19, in which said primers are selected to specifically amplify a nucleic acid product of the IL-10 gene.

65. The method of claim 24, wherein the quantity of IL-10 polypeptide in peripheral blood is measured using an *in vitro* bioassay that detects at least one IL-10 mediated biological process.

~~66.~~ A method for treating a subject with cancer comprising the steps of:

(i) providing an antisense expression construct comprising a nucleic acid, wherein the RNA expressed from said nucleic acid binds under high stringency conditions to an RNA product of the IL-8 or IL-10 genes, and a promoter functional in eukaryotic cells wherein said nucleic acid is under transcriptional control of said promoter; and

- (ii) contacting said expression construct with peripheral leukocyte cells of said subject in a manner that allows the uptake of said expression construct by said cells.

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67. The method of claim 66, wherein expression of said nucleic acid results in the modification of the immune response of said peripheral leukocyte cells to said cancer.

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68. A kit according to claim 31, comprising a pair of primers selected to amplify a nucleic acid sequence encoded by an IL-10 gene.

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69. The method of claim 39, wherein the level of IL-10 in a biological sample is measured using at least one antibody that binds to an IL-10 gene product.

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70. The method of claim 69, wherein the level of IL-10 gene product bound to antibody is measured by ELISA.

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71. The method of claim 39, wherein the level of IL-10 in a biological sample is measured using at least one oligonucleotide probe that binds an IL-10 messenger RNA (mRNA).

~~72.~~ An isolated nucleic acid segment having a sequence consisting essentially of SEQ ID NO:49 or its complement.

55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000 1001 1002 1003 1004 1005 1006 1007 1008 1009 1010 1011 1012 1013 1014 1015 1016 1017 1018 1019 1020 1021 1022 1023 1024 1025 1026 1027 1028 1029 1030 1031 1032 1033 1034 1035 1036 1037 1038 1039 1040 1041 1042 1043 1044 1045 1046 1047 1048 1049 1050 1051 1052 1053 1054 1055 1056 1057 1058 1059 1060 1061 1062 1063 1064 1065 1066 1067 1068 1069 1070 1